FIGURE 1A

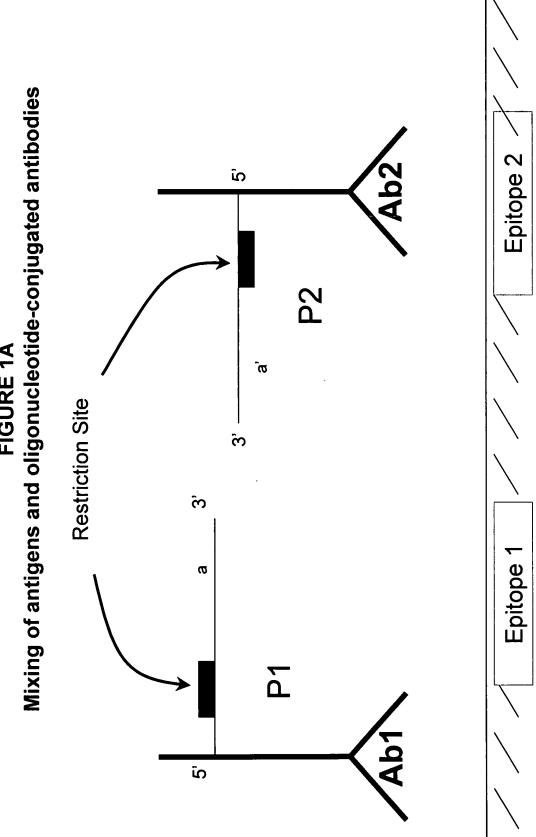
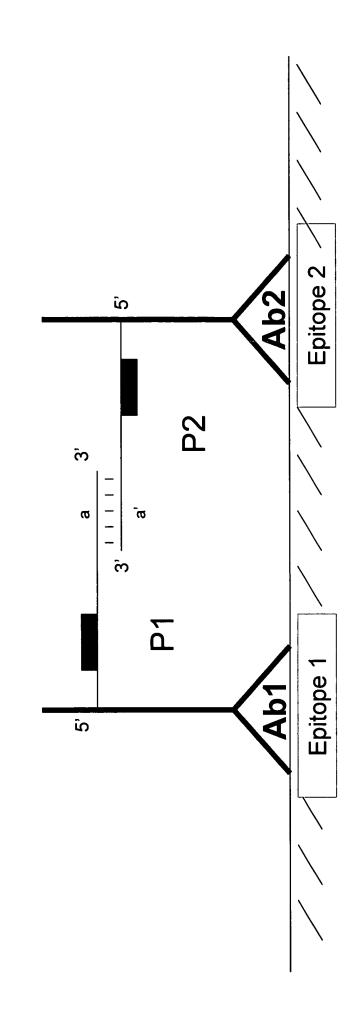
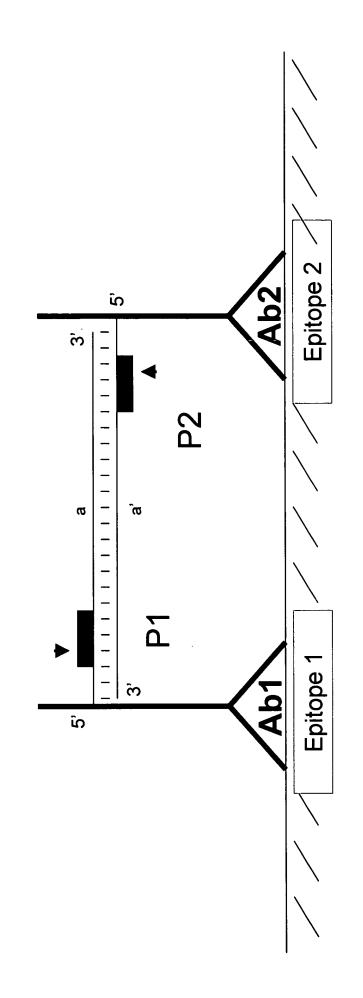


FIGURE 1B
Hybridization of adjacent oligonucleotide probes

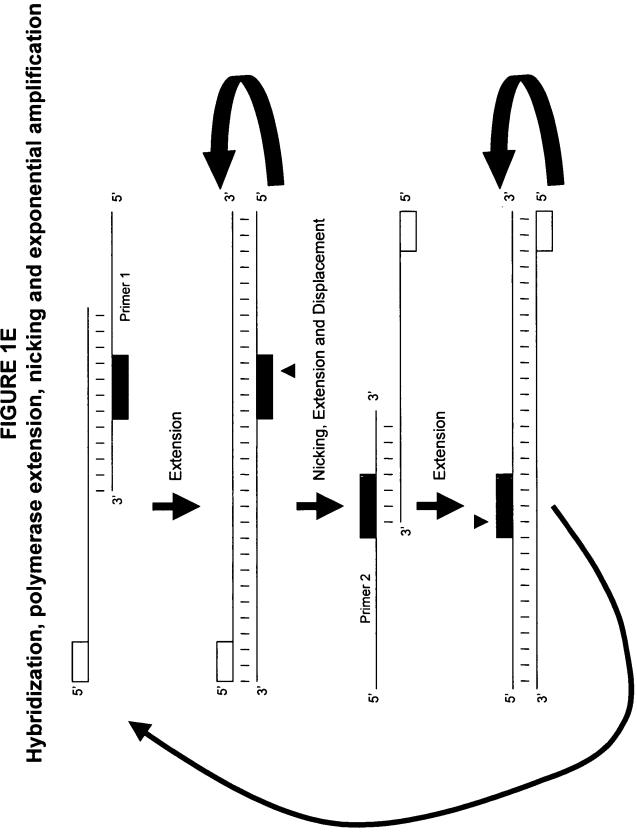


Polymerase extension and restriction enzyme nicking **FIGURE 1C**



Extension, displacement and linear amplification Epitope 2 Ω က် ູນ 2 ري کا_ ດິ က **FIGURE 1D** ์ซ $\boldsymbol{\sigma}$ 5 **№**5'₁ က် $\hat{\omega}$ ₹5, က် 5 Epitope 1 က် Ab1 Ω Restriction Site Partial

FIGURE 1E



Mixing of antigens and oligonucleotide-conjugated antibodies **FIGURE 1F**

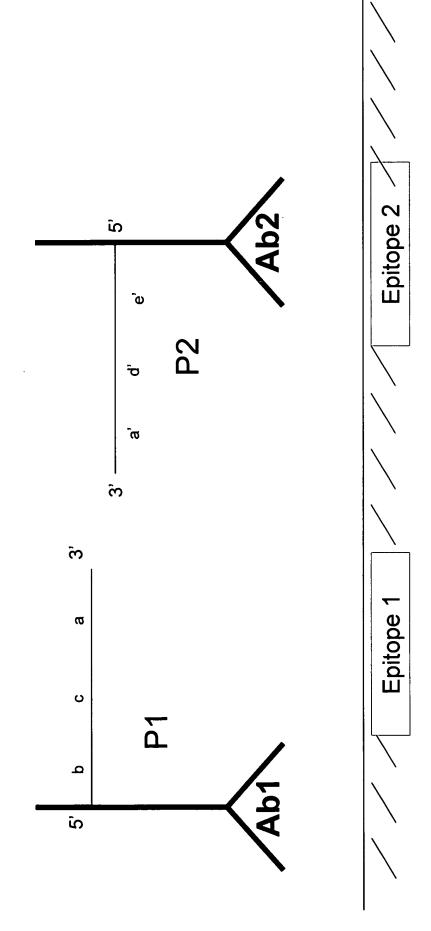


FIGURE 1G
Hybridization of adjacent oligonucleotide probes

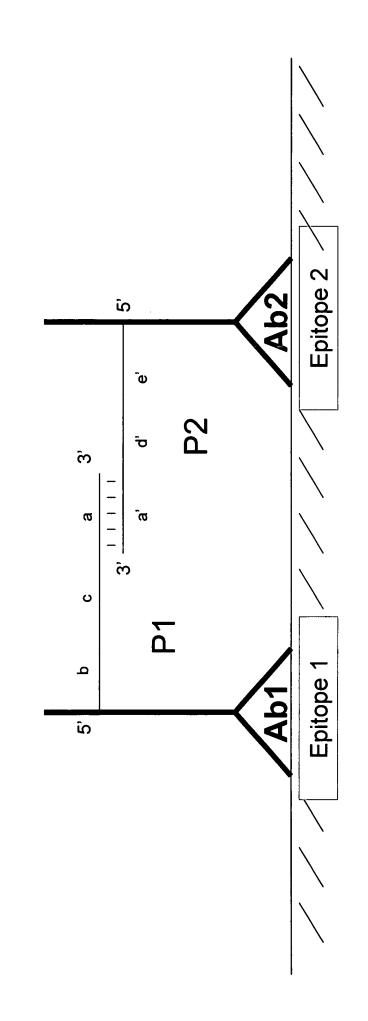
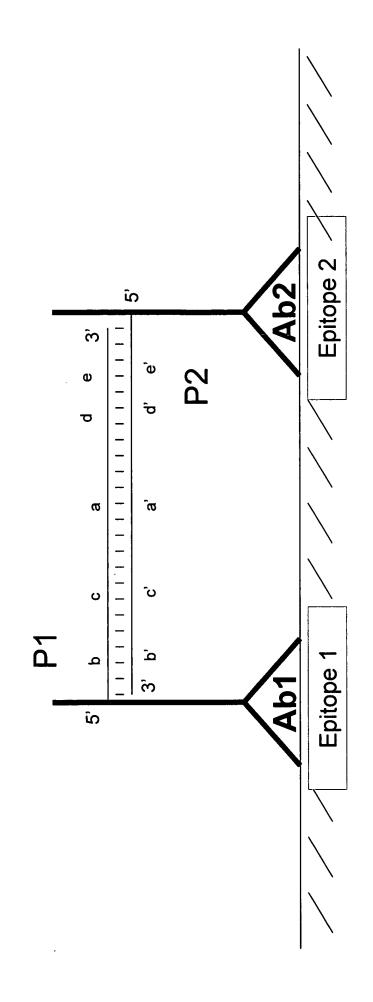
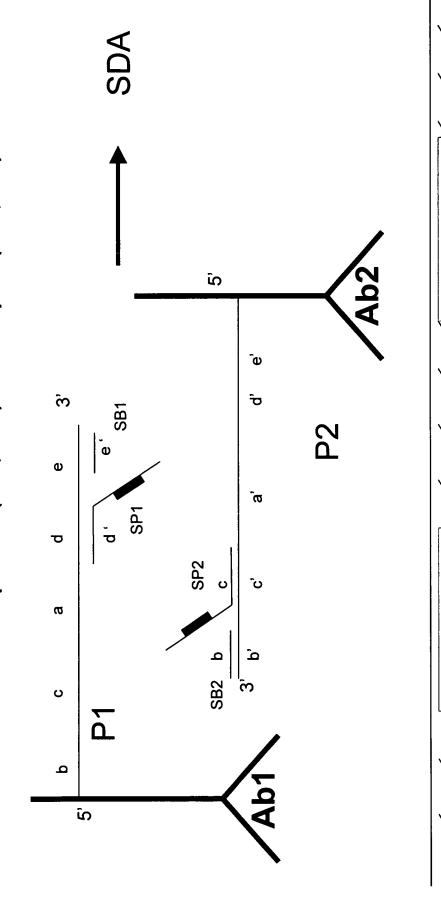


FIGURE 1H
Extend oligonucleotide probes with polymerase



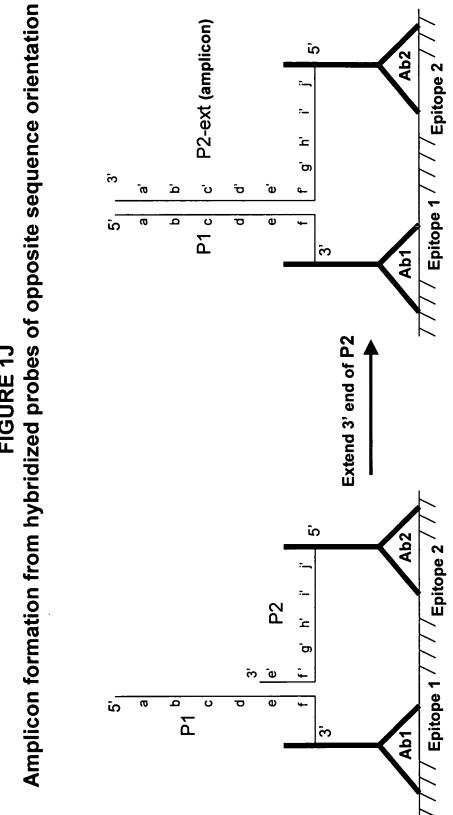
Denature probe-extension duplex and bind SDA primers (SP1, SP2) and bumpers (SB1,SB2) **FIGURE 1I**



Epitope 2

Epitope 1

FIGURE 1J



Hybridization of splint oligonucleotide **FIGURE 2A**

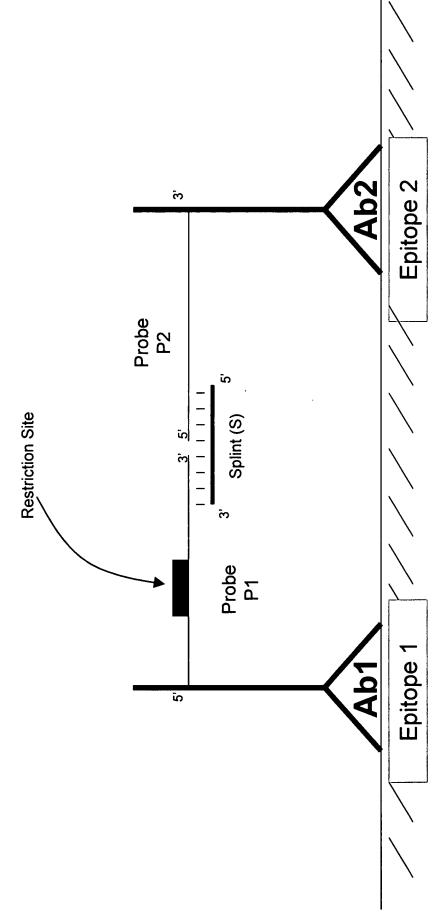


FIGURE 2B Ligation of adjacent oligonucleotide probes

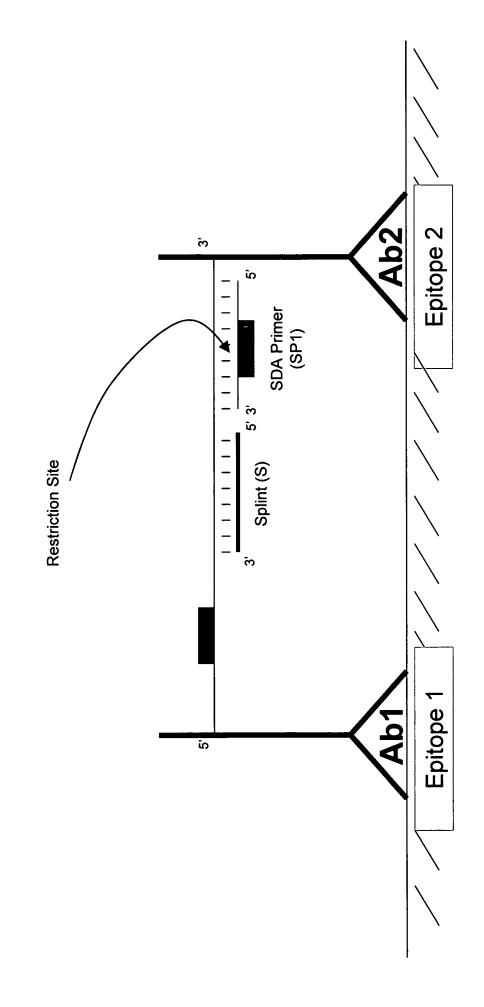


FIGURE 2C
DNA polymerase extension and displacement

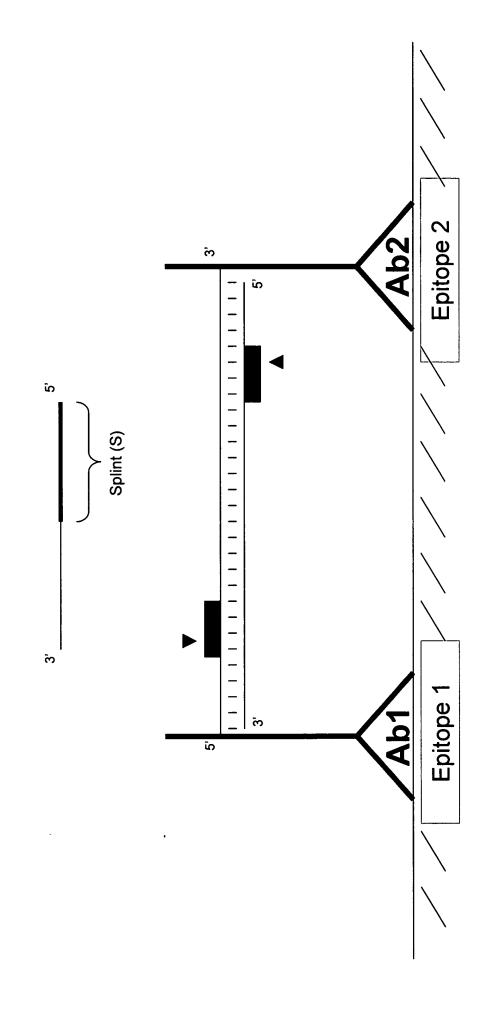
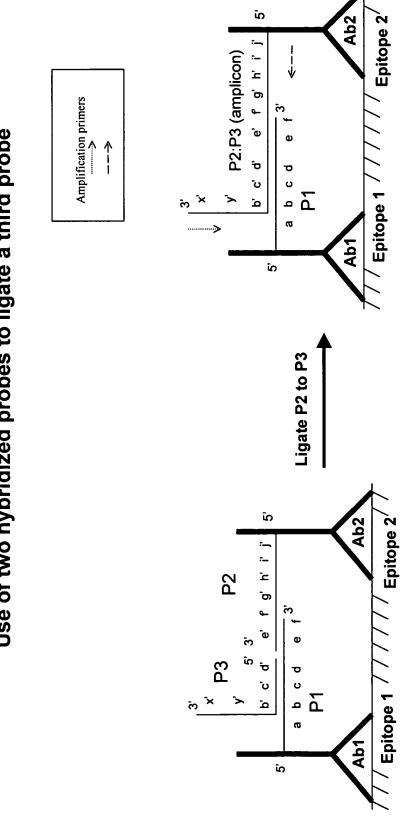


FIGURE 2D
Use of two hybridized probes to ligate a third probe



Use of two hybridized probes in opposite sequence ڡٛ ပ် σ œ[¯] σ P1 c ີດ Ø ρ Ð orientation to ligate a third probe **FIGURE 2E** 3' P2 (not amplifiable) P3 ڡٛ ัต ດ໌ ပ က် ີດ Ø Ω ပ Φ 7

P2:P3 (amplifiable) Amplification primers û Epitope 2 È ້ ວາ Epitope 1 / က် Ab1 Ligate P2 to P3 ດ໌ Epitope 2 <u>`</u>_ <u></u>כ Epitope 1 / က Ab1

Epitope 2 Ab2 Ω Sequence slh-2 Primer က် Probe - P2 က Detector Region Sequence srh-1 Probe-P1 Primer **Tether Oligonucleotide** Restriction Site Ŝ ဥ Epitope 1 ŝ

FIGURE 3A Single-tether oligonucleotide

Epitope 2 Ab2 Ω က် Single-tether oligonucleotide: extension and displacement Primer slh-2 Detector Region Primer srh-1 **FIGURE 3B** Restriction Site ີ່ດ က် Epitope 1 က် A_b1 ດົ

SDA Primer SP2 က် $\mathbf{\omega}$ Nicking, extension, displacement and capture **Polymerase** Primer 2 Nick, Extend, Displace ĝ Nick and Displace û က် Detector Region Primer 2 Sequence Primer 1 Sequence Restriction Site ດ໌

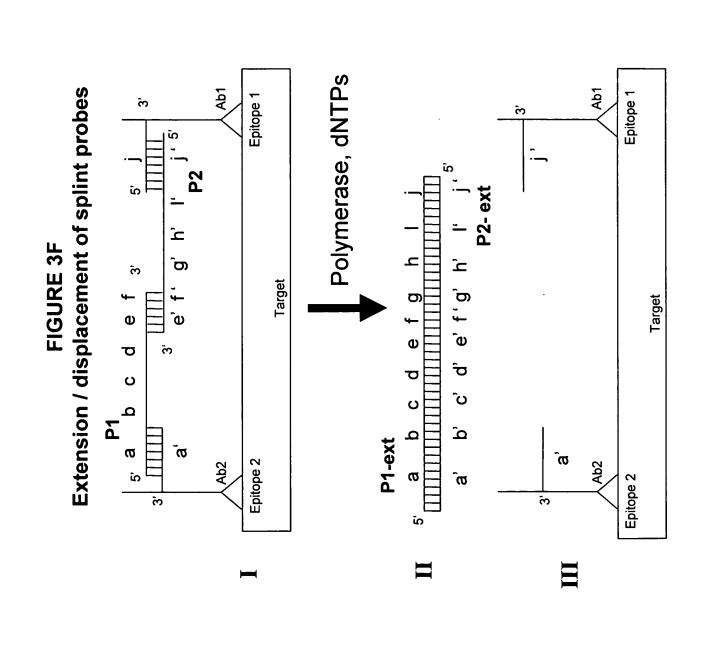
FIGURE 3C

Nick, Extend, Displace Polymerase Nicking, extension, displacement and capture (cont.) Exponential G щ SDA Primer, SP1 FIGURE 3D Primer 1 Ш

Amplification

Epitope 2 က Ω ري ا <u>۔</u> , თ က် Φ က Q ပ Ω ũ 7 ัต ത Epitope 1 Ω က်

FIGURE 3E Splint probes (3'/3' configuration)



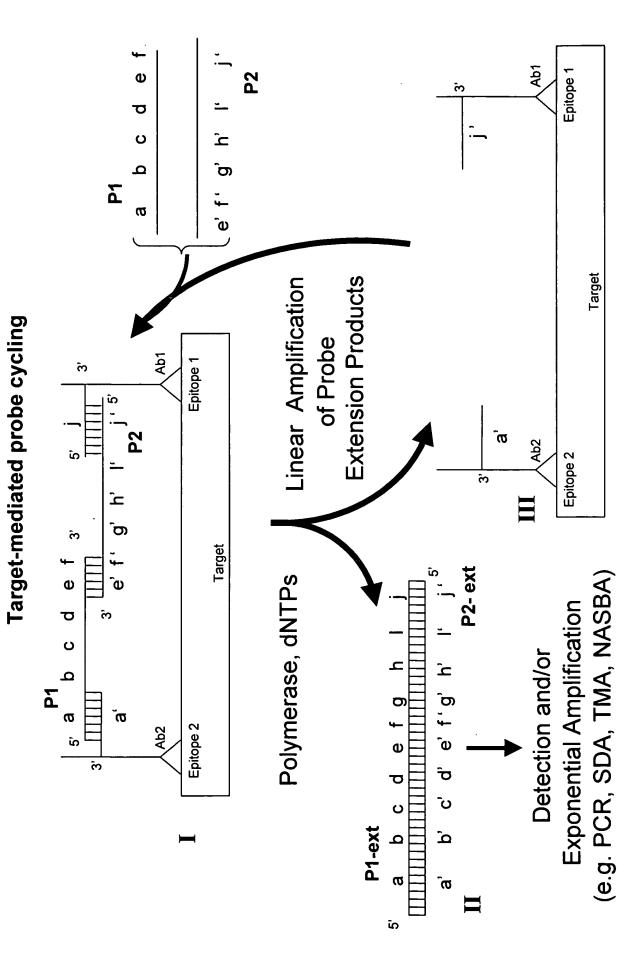
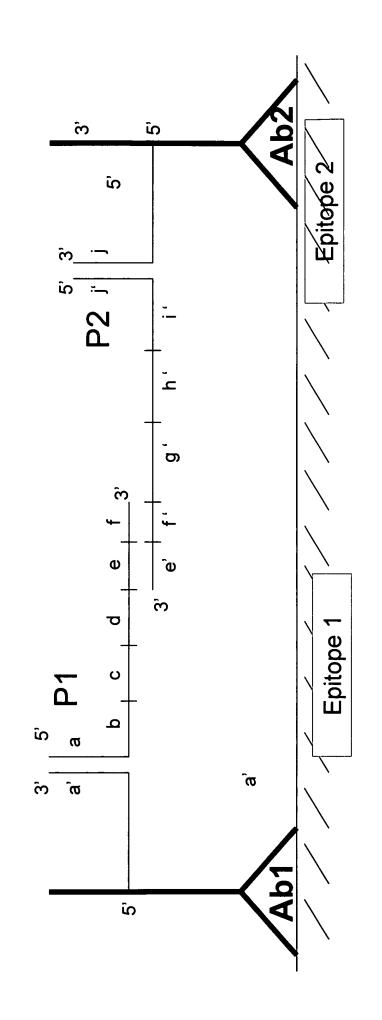


FIGURE 3G

က Epitope 2 ũ <u>,</u> D က Φ က် O Epitope 1 ပ Ω Ω σ ື ຫ ัต က ũ

FIGURE 3H Splint probes (5'/3' configuration)

FIGURE 3I Splint probes (5'/5' configuration)



| Epitope 2 ũ က **P2** က် Ω Splint probes (3'/3' configuration) <u>,</u> Ligation splint ຸ ດ D Ω ب ع Φ Ū က် ᠣ ပ Ω Ŵ 7 **ື** ຫ σ Epitope 1 ũ က်

FIGURE 3J

Epitope 2 Ω က | splint ~ က် ດິ FIGURE 3K Splint probes (3'/3' configuration) **P2** ũ _ ත **4** 33 Φ က် ס ပ Ω ΩĨ 7 ֿס $\boldsymbol{\omega}$ Epitope 1 Ω Ab1 က

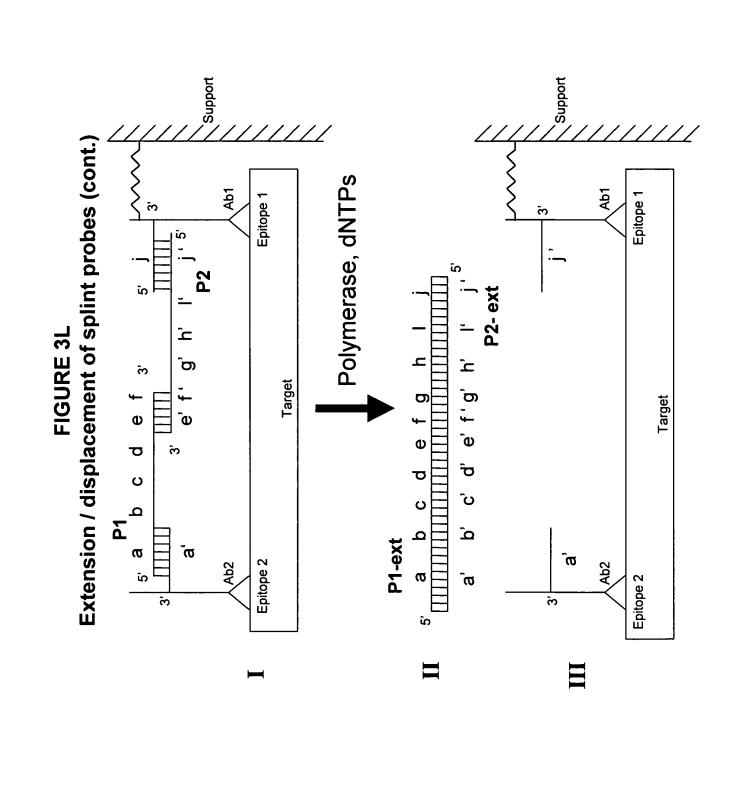
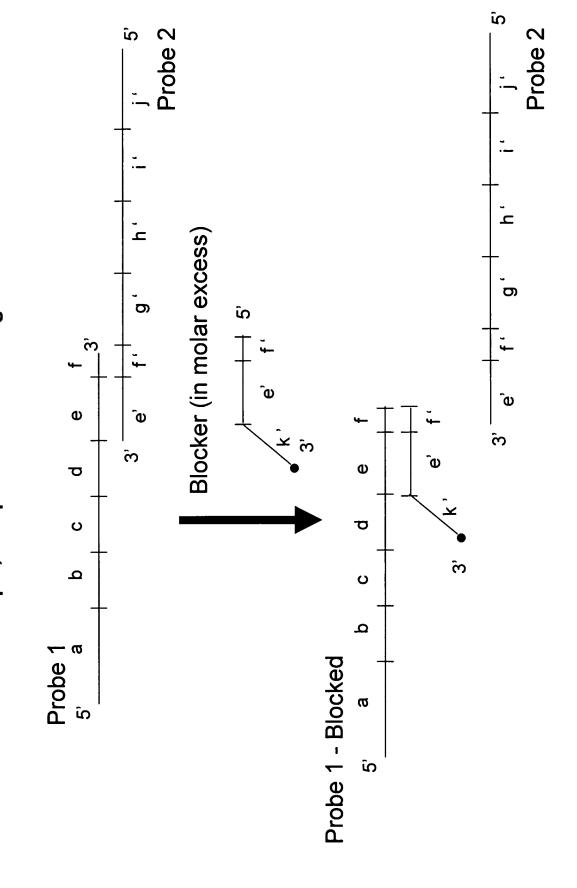
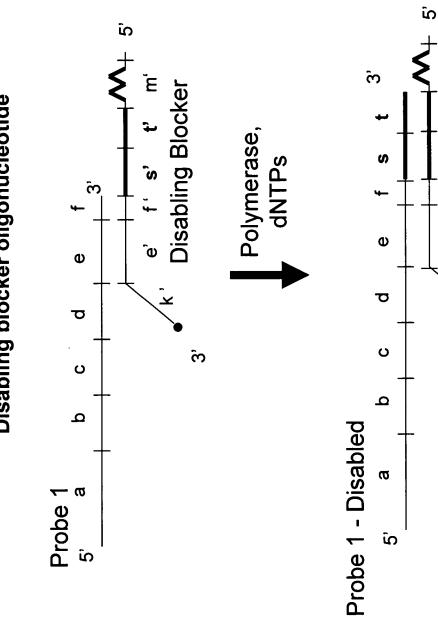


FIGURE 4A Simple, competitive blocker oligonucleotide



ດີ ũ Probe 2 Probe 2 Recessed, competitive blocker oligonucleotide Blocker (in molar excess) b D ω **FIGURE 4B** œ[^] Φ က် က် Φ σ Ū က ပ Ω ပ က် ۵ Probe 1 Probe 1 - Blocked ത Ω Ω

FIGURE 4C
Disabling blocker oligonucleotide



œ

က်

FIGURE 4D
Displaceable blocker oligonucleotide

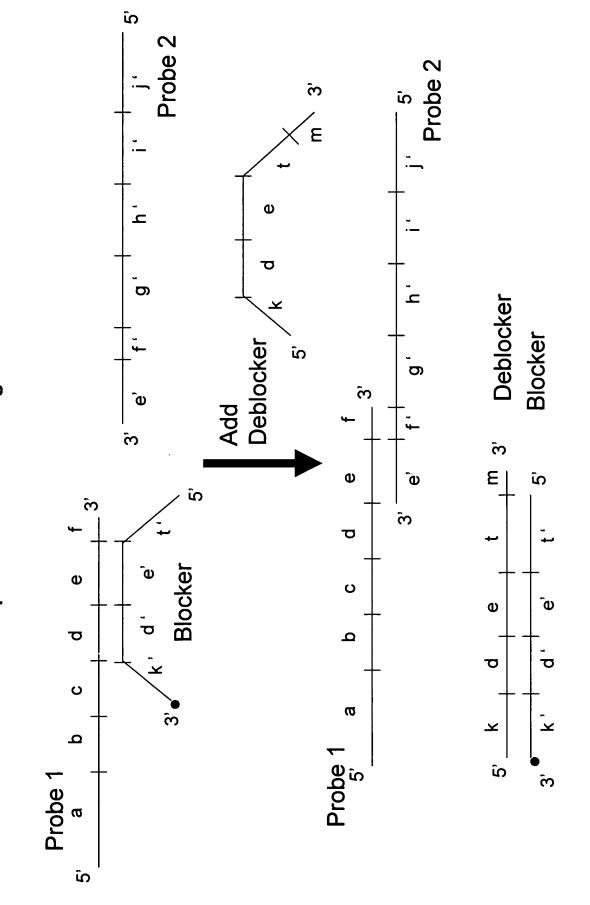
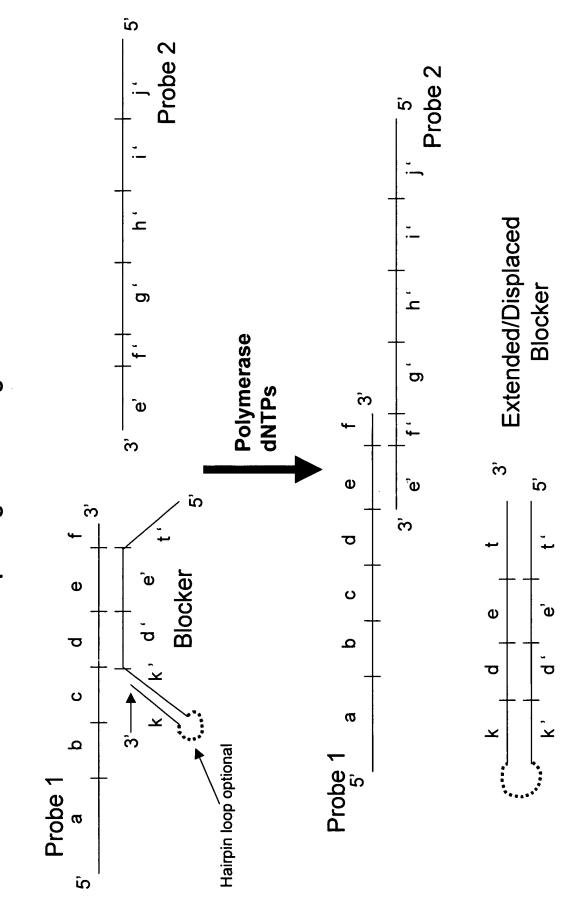
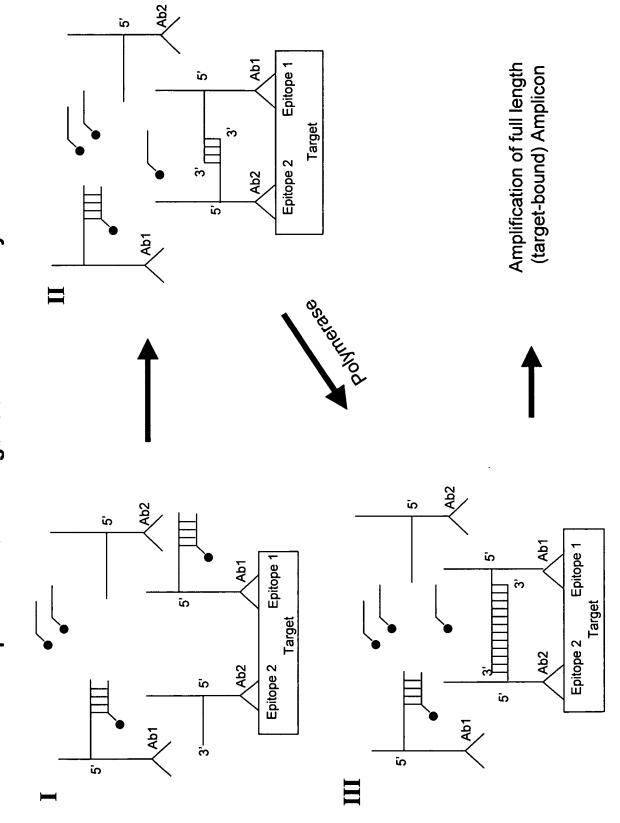


FIGURE 4E Self-displacing blocker oligonucleotide



Ω Probe 2 ũ Probe 2 Ω Use of 3' probe tail to stabilize probe-blocker duplex Ň ຸດ Ň က် က D D **FIGURE 4EE** × œ[¯] Ū Φ ω **Blocker** က် က် Œ Φ Q ပ $\boldsymbol{\sigma}$ Ω ပ ۵ Probe 1 Probe 1 - Blocked σ Ω ũ

Competitive blocker oligonucleotide in binary immuno-SDA **FIGURE 4F**



Disabling blocker oligonucleotide in binary immuno-SDA **FIGURE 4G**

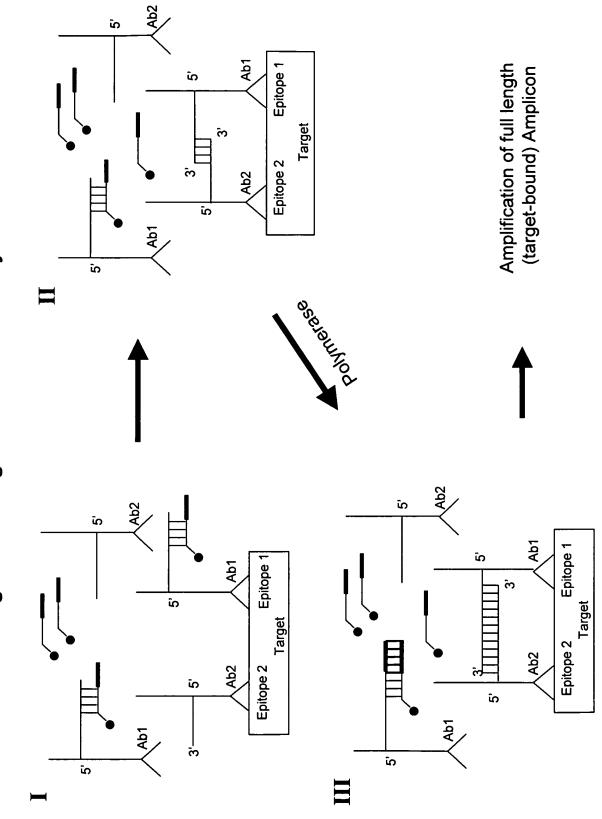
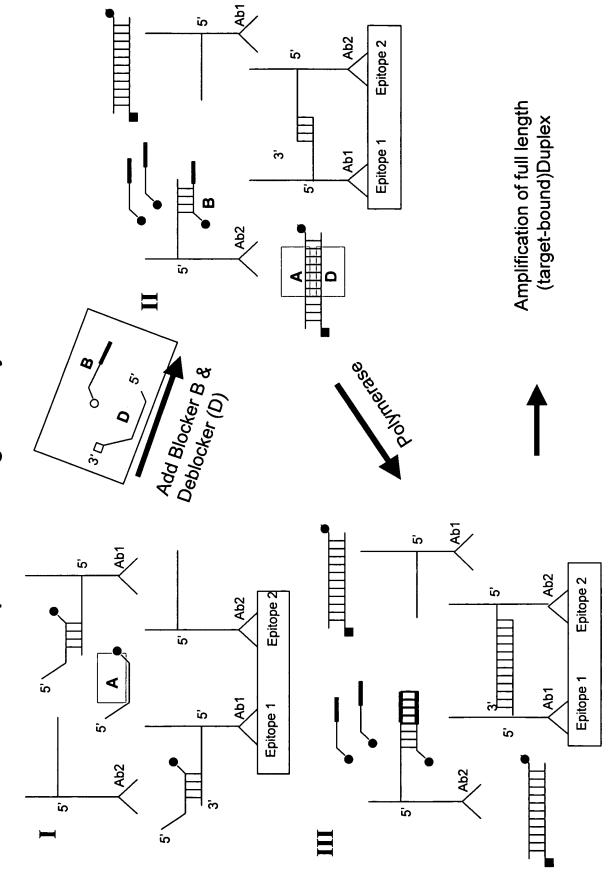
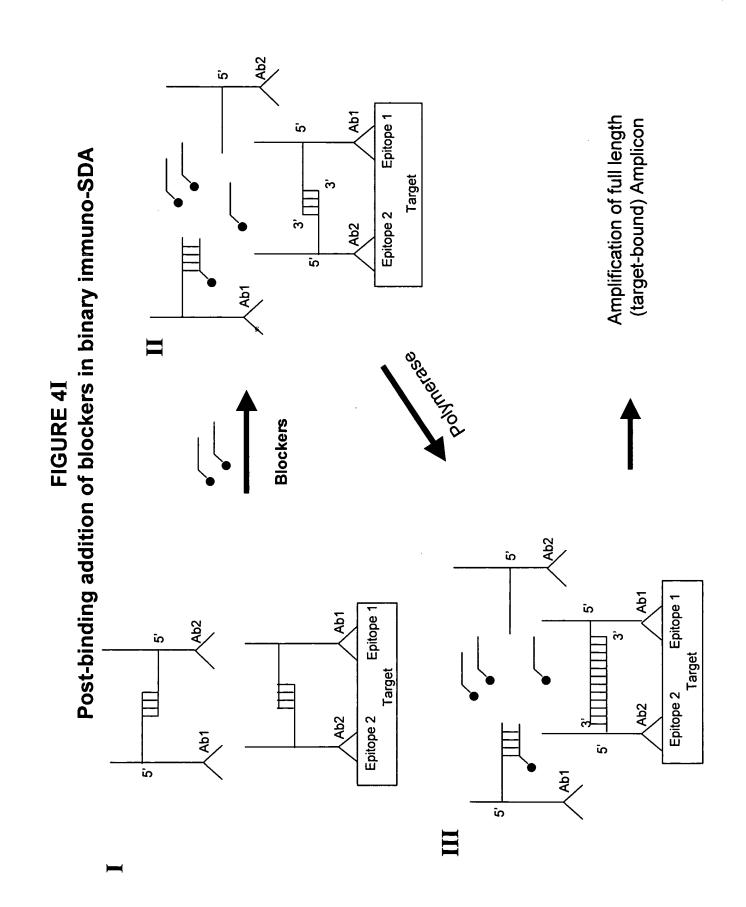


FIGURE 4H
Step-wise blocking in binary immuno-SDA





Epitope 2 Ab2 Ω Probe (P2) Sequence 1 Primer က် ။ က Detection Region Splint (S) Sequence 2 Primer RNA Polymerase Promoter ũ Probe (P1) ີທ Epitope 1 Ab1 Ś

FIGURE 5A Splint oligonucleotide hybridization

Epitope 2 Ab2 Ω က် Sequence 1 Primer Detection Region Primer Sequence 2 RNA Polymerase Promoter ູ່ດ 'n Epitope 1 က် A_b1 ດ໌

FIGURE 5B Extension and displacement

RNA polymerase activity, hybridization and extension **FIGURE 5C**

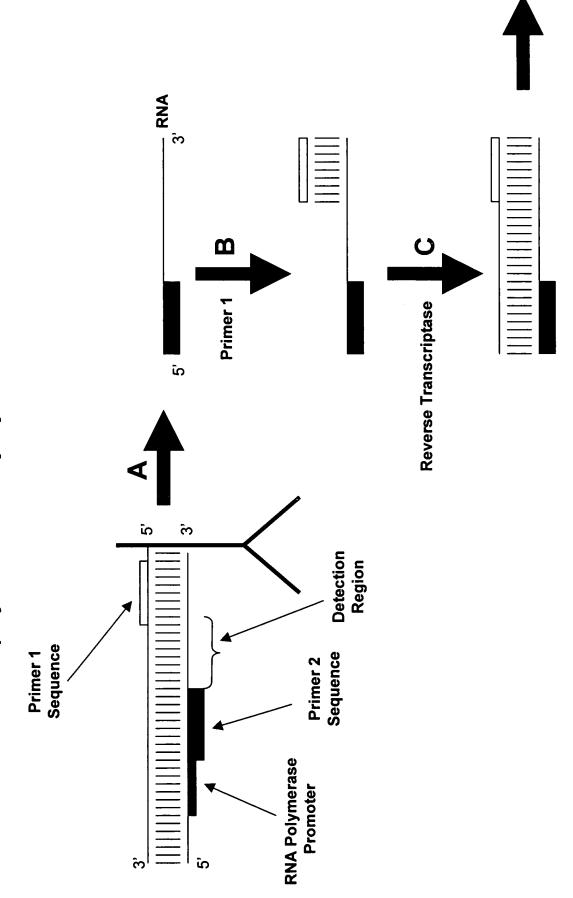


FIGURE 5D RNase H activity, hybridization and extension

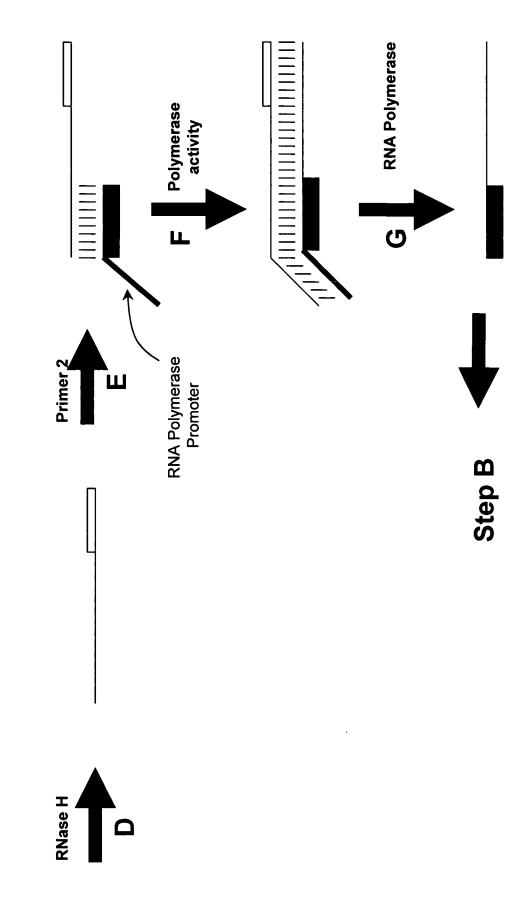
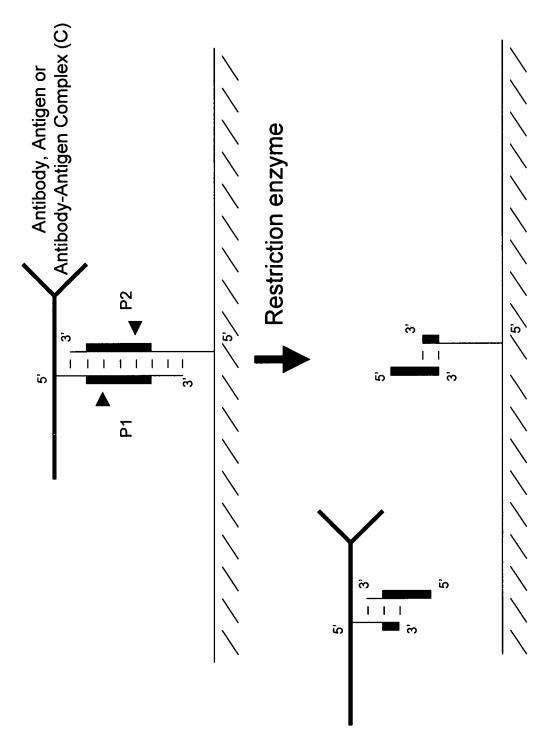


FIGURE 6A Restriction endonuclease-mediated release

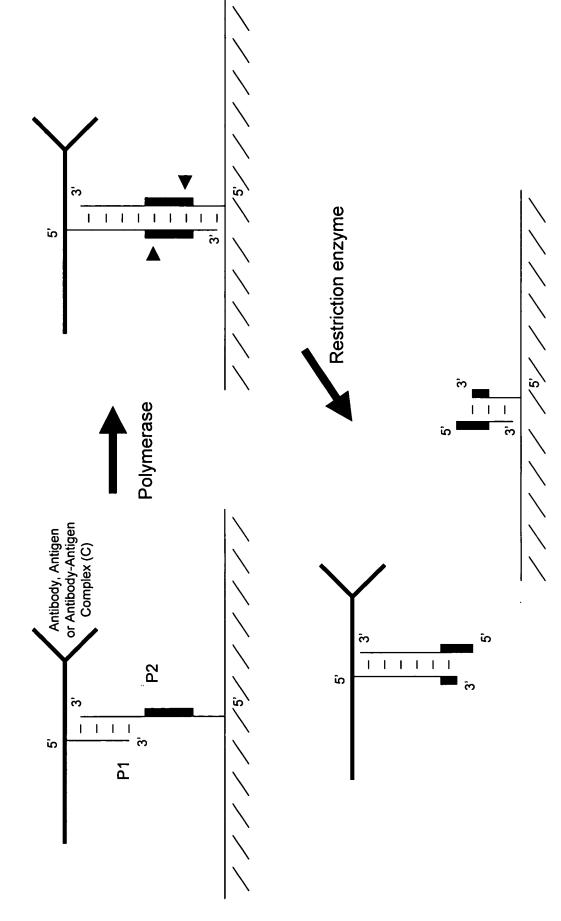


1 1 ີທ ۵. ۵. က် Displacement oligonucleotide Restriction endonuclease-mediated release ດົ က် Antibody, Antigen or Antibody-Antigen Complex (C) Restriction enzyme ŝ <u></u>ב က် P2 I I I Iົດ က် P4 ດ໌

FIGURE 6B

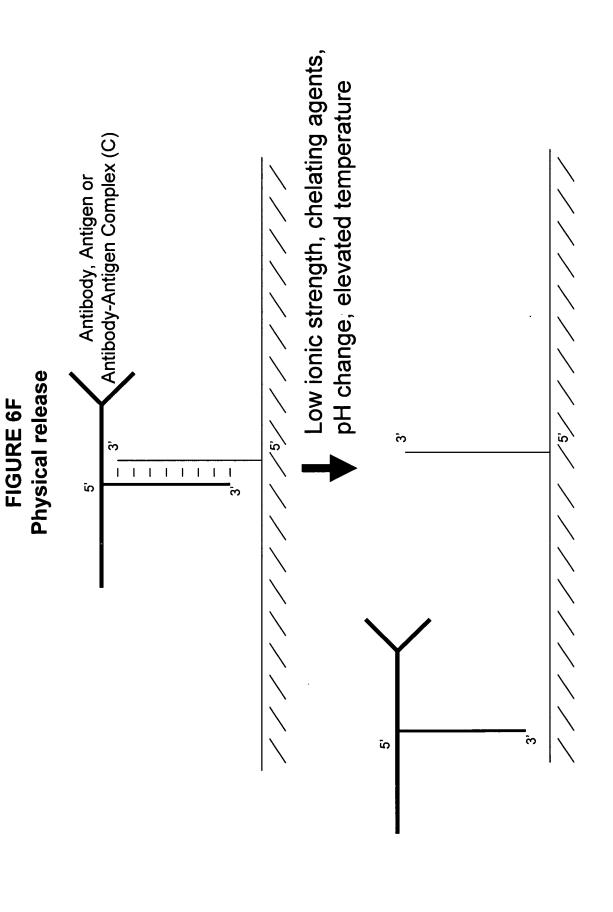
Antibody, Antigen or Antibody-Antigen Complex (C) Restriction enzyme Restriction endonuclease-mediated release **FIGURE 6C** <u>2</u>2 ■ က ີ່ເດ <u>g</u> က

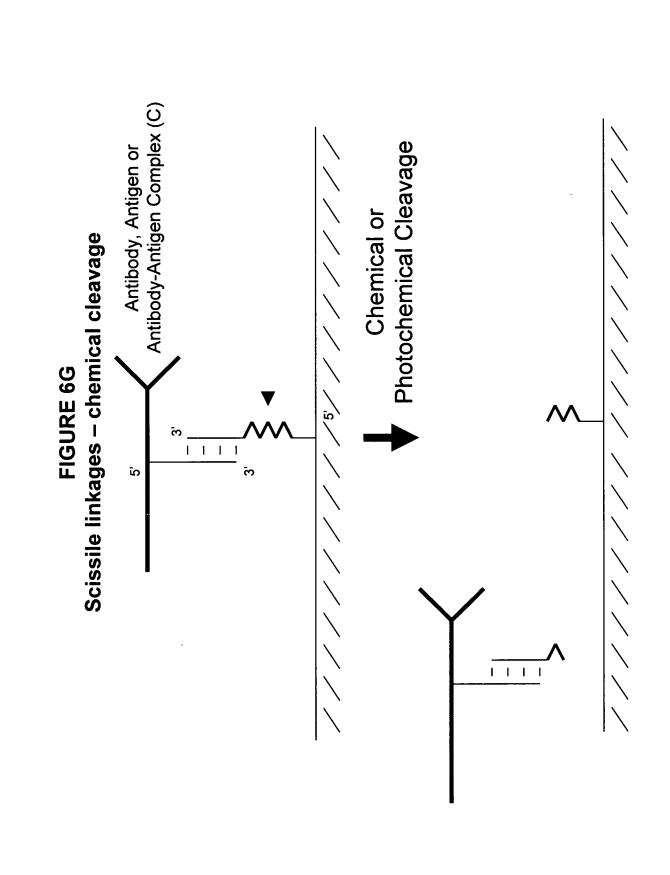
Polymerase and restriction endonuclease-mediated release **FIGURE 6D**

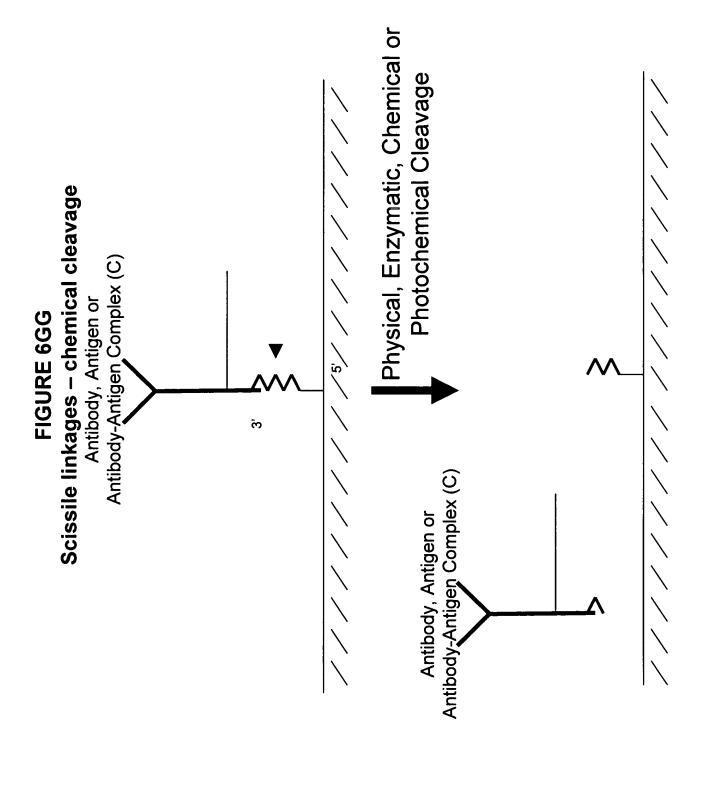


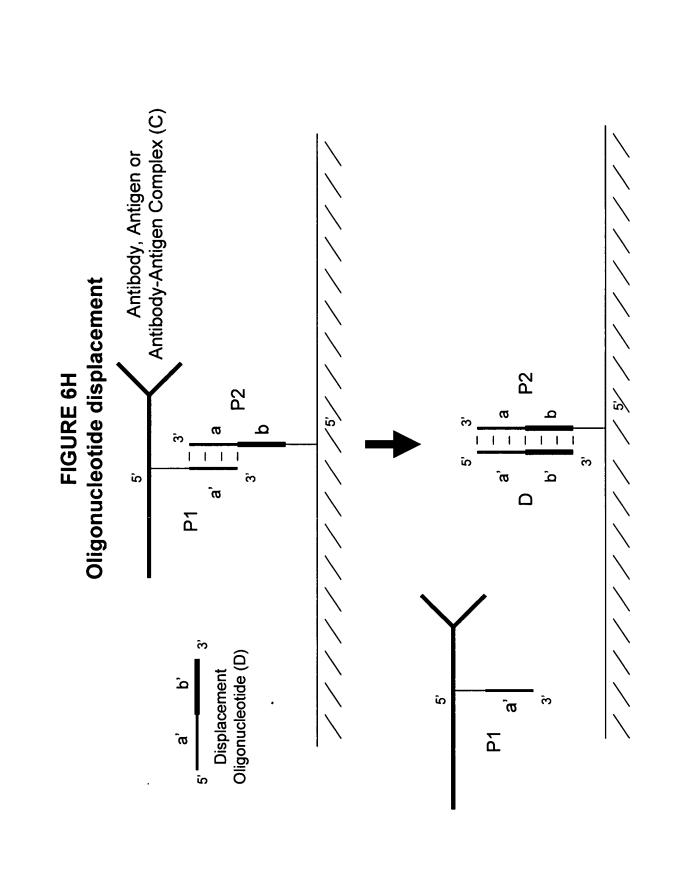
Polymerase and restriction endonuclease-mediated release Restriction enzyme ا <u>ا</u> ش ດ໌ FIGURE 6E Polymerase ດີ Antibody, Antigen or Antibody-Antigen Complex (C) ດົ **P**2 5, က် ı 1 1 'n ດ໌ 7

ı 1 ດົ









. 5' **P**2 , 0 σ 'n က ດ໌ ື້ຫ 7 Strand Displacing Polymerase Oligonucleotide extension Hybridization P2 Antibody, Antigen or Antibody-Antigen Complex (C) ത ດ໌ 1 က် ڡٛ ัต P2 Ø $\mathbf{I} \cdot \mathbf{I}$ 1 - [<u>რ</u> ົດ **ີ** ຕ 7 ດ໌ ັດ 7 Displacement Oligonucleotide (D) ŝ ۵

FIGURE 6I

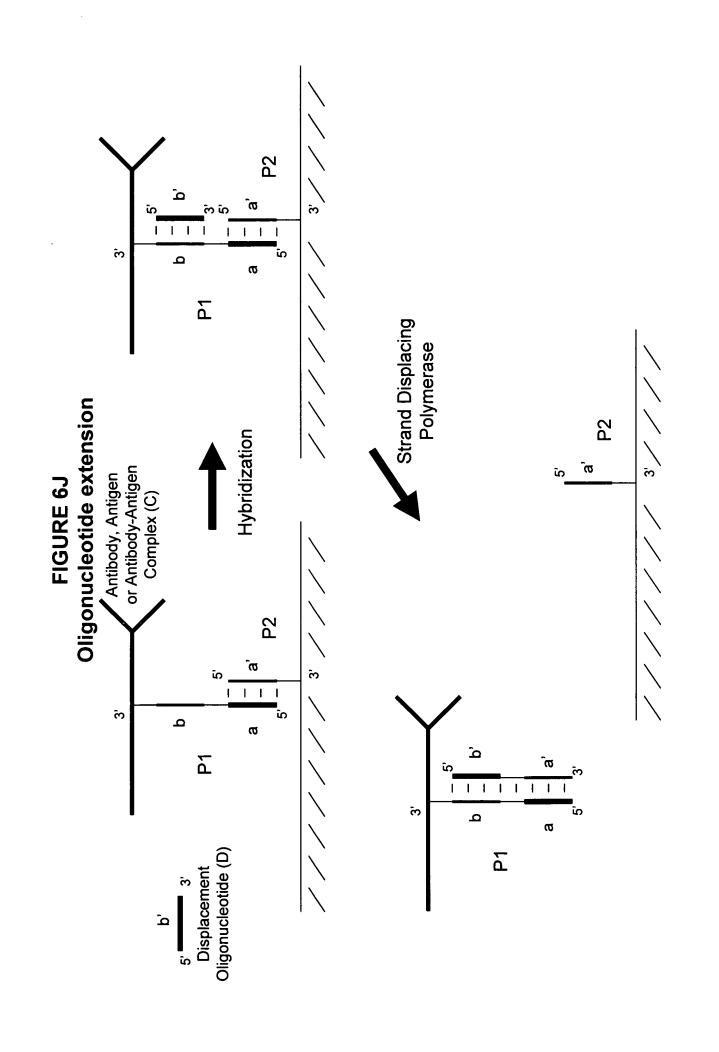
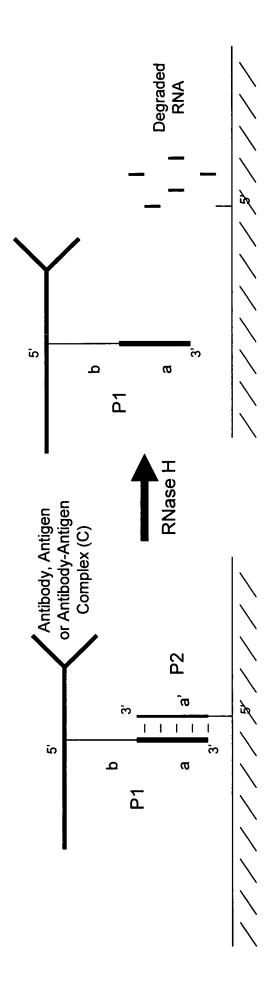


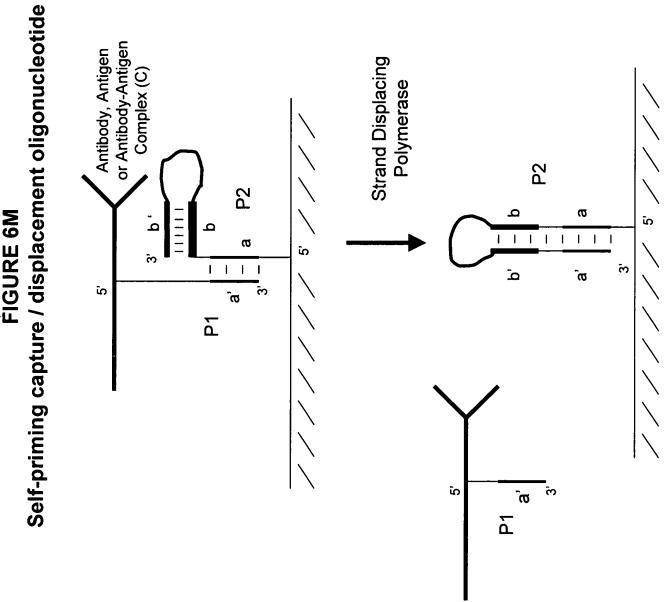
FIGURE 6K RNase H release

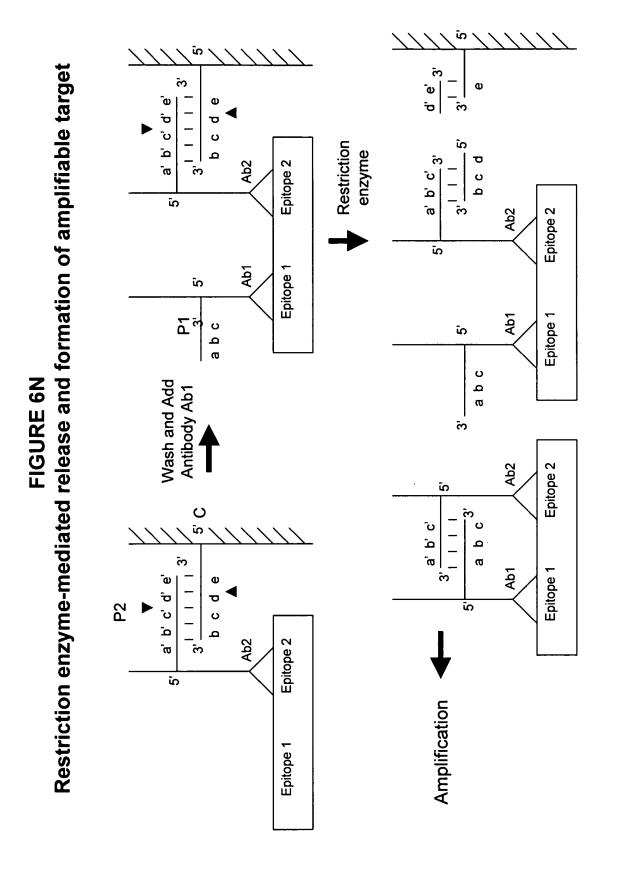


P2 ์ต Ω Degraded RNA Φ RNase H Antibody, Antigen or Antibody-Antigen Complex (C) **P**2 σ 1 1 1 1 1 ົດ ത Ω 7

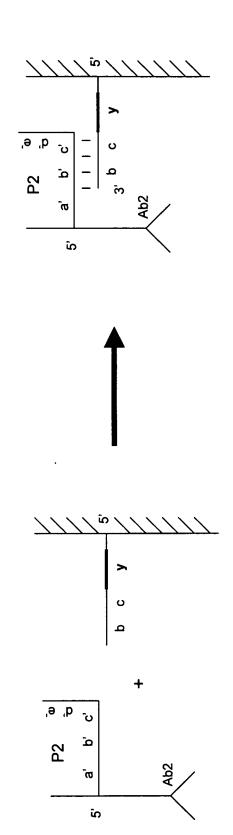
FIGURE 6L RNase H release

FIGURE 6M

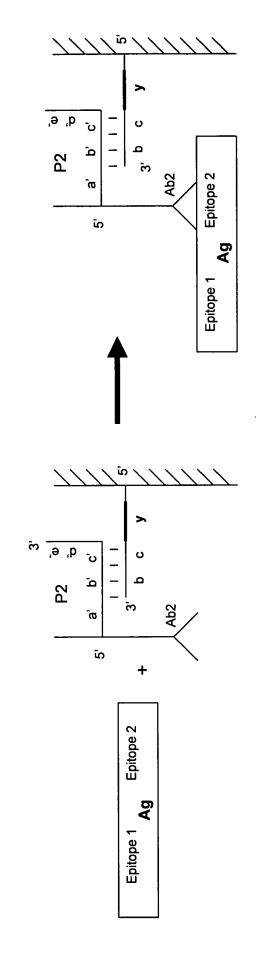




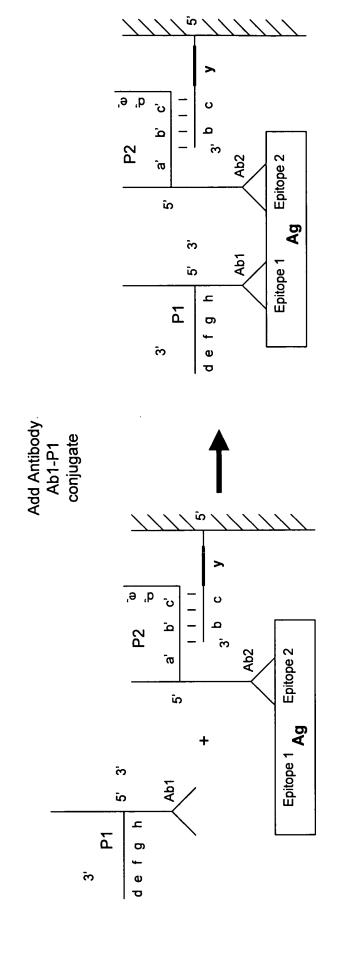
Immobilization of antibody-probe conjugate by hybridization of a probe oligonucleotide to a capture oligonucleotide **FIGURE 7A**



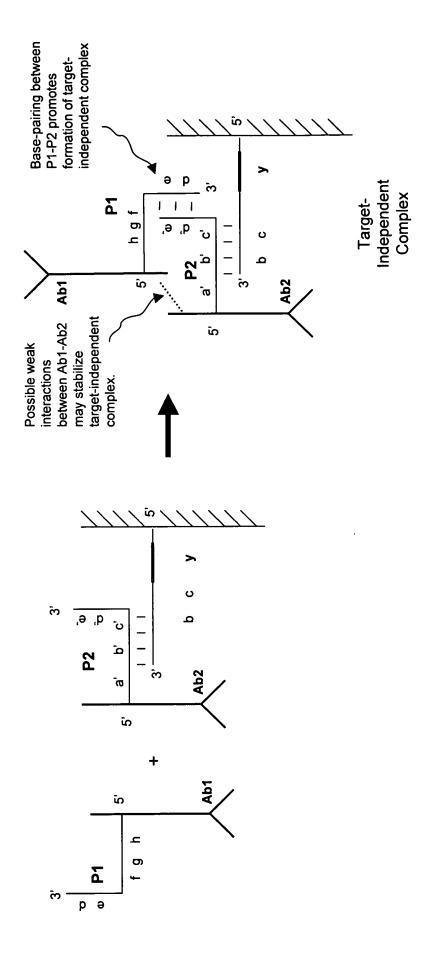
Binding of target ligand to antibody-probe conjugate immobilized by a capture oligonucleotide **FIGURE 7B**



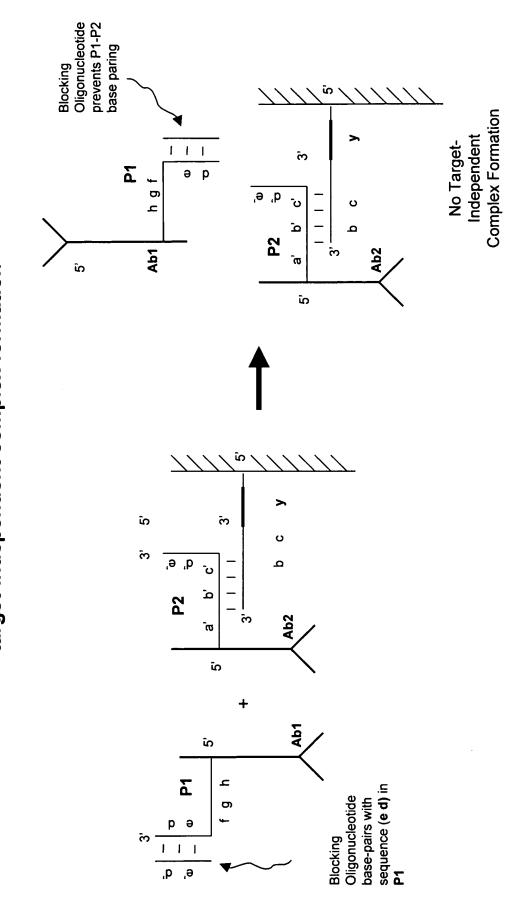
Formation of immobilized two-site "sandwich" complex by binding second antibody-probe conjugate to target ligand **FIGURE 7C**



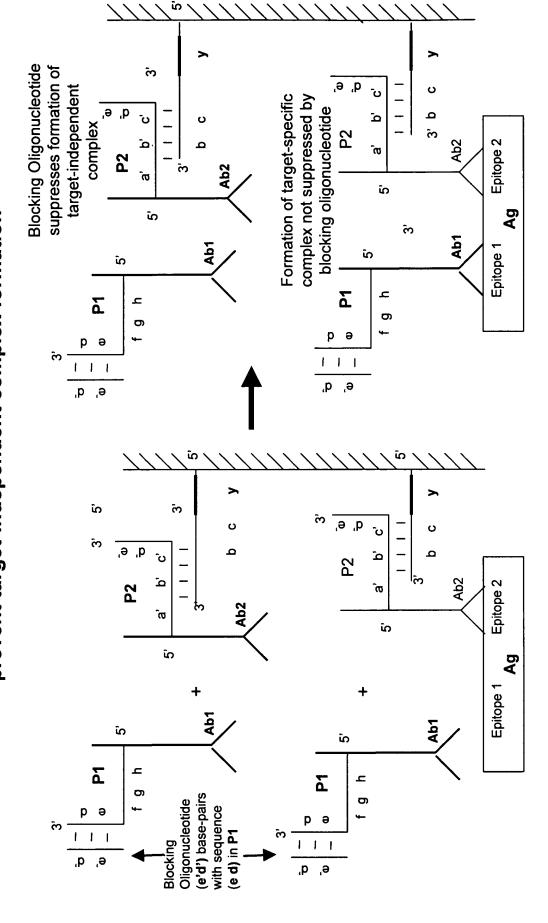
Formation of target-independent complex involving probe-probe (P1-P2) interactions **FIGURE 7D**



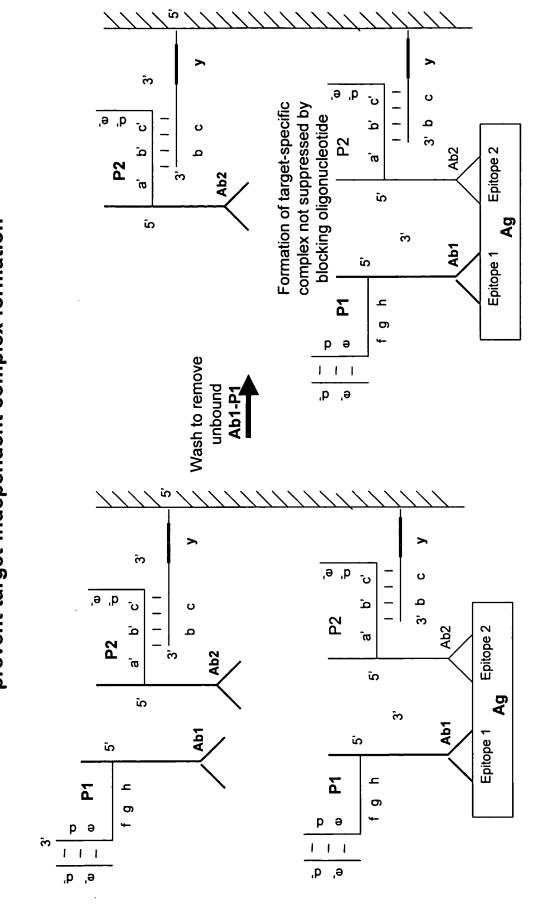
Use of blocking oligonucleotide to suppress P1-P2 interactions leading to target-independent complex formation **FIGURE 7E**



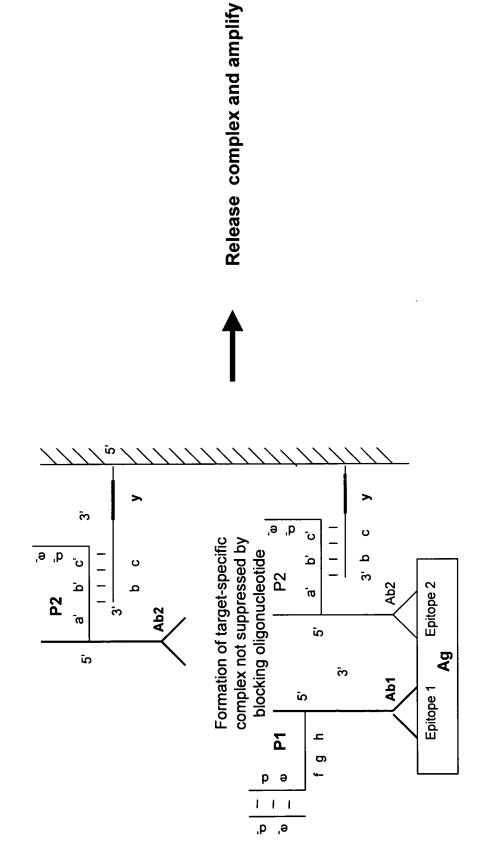
Use of blocking oligonucleotide to suppress P1-P2 interactions and prevent target-independent complex formation **FIGURE 7F**

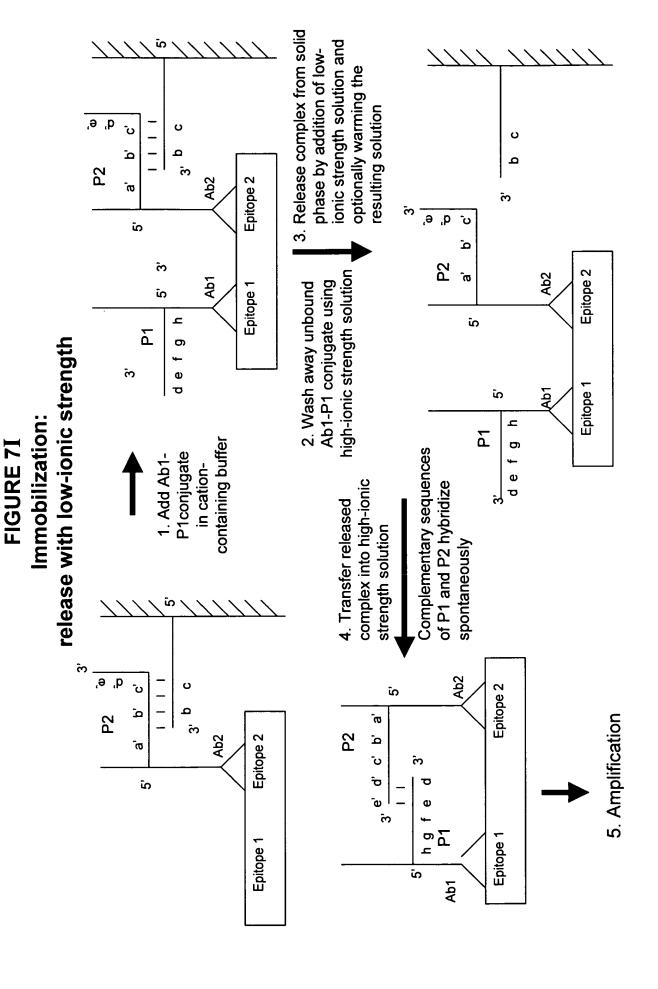


Use of blocking oligonucleotide to suppress P1-P2 interactions and prevent target-independent complex formation **FIGURE 7G**

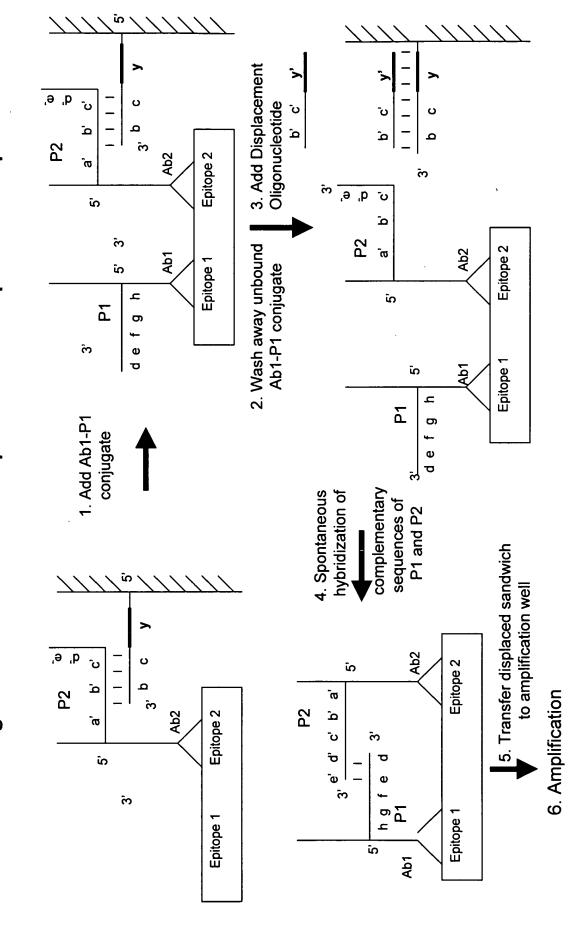


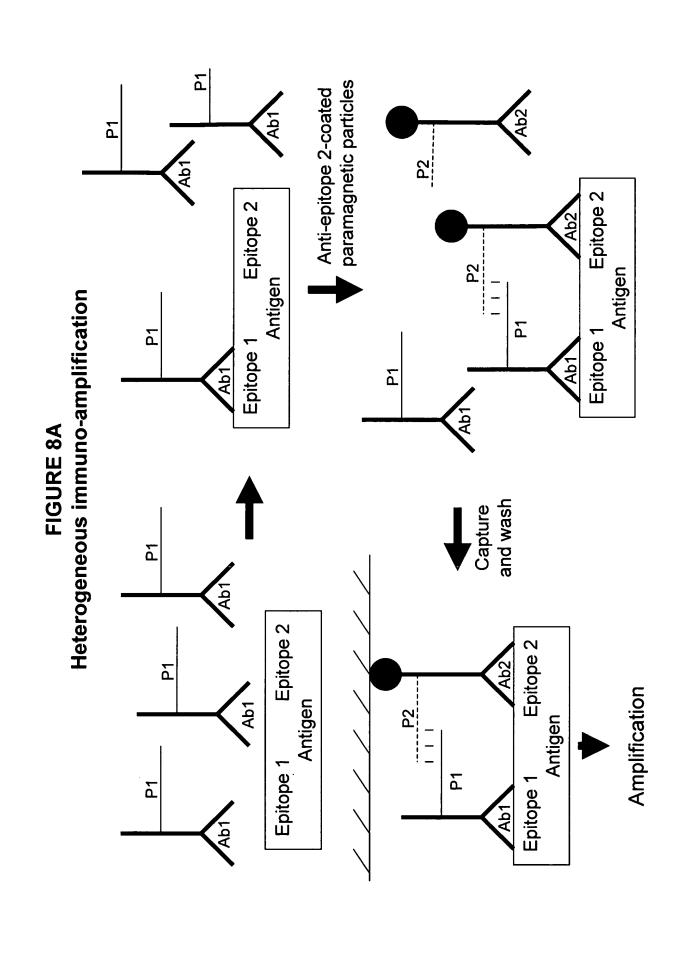
Use of blocking oligonucleotide to suppress P1-P2 interactions and prevent target-independent complex formation **FIGURE 7H**

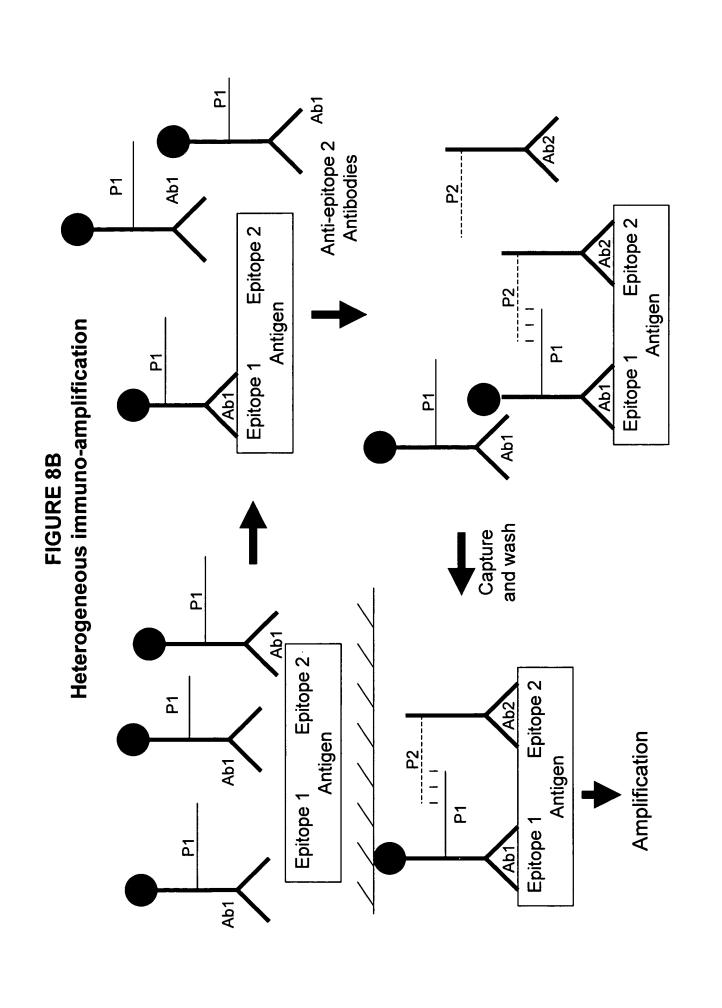




Heterogeneous formation and displacement of amplifiable complex **FIGURE 7J**



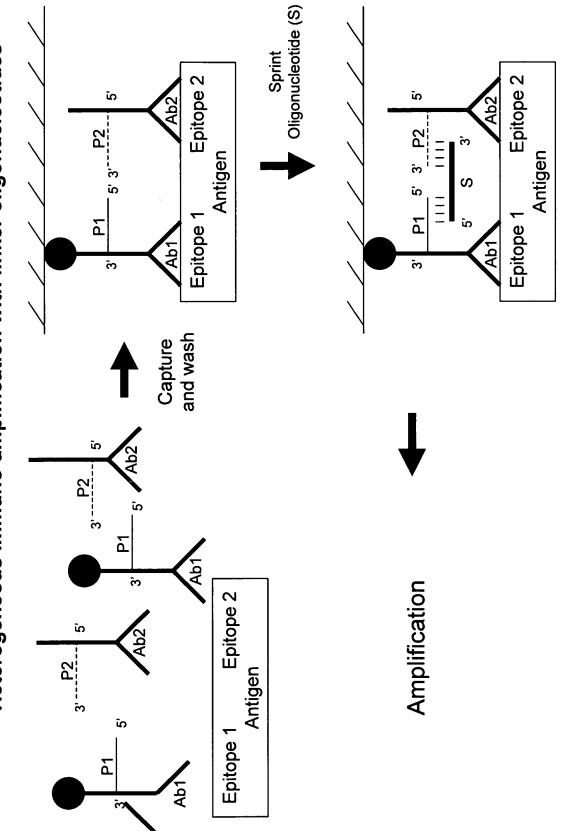




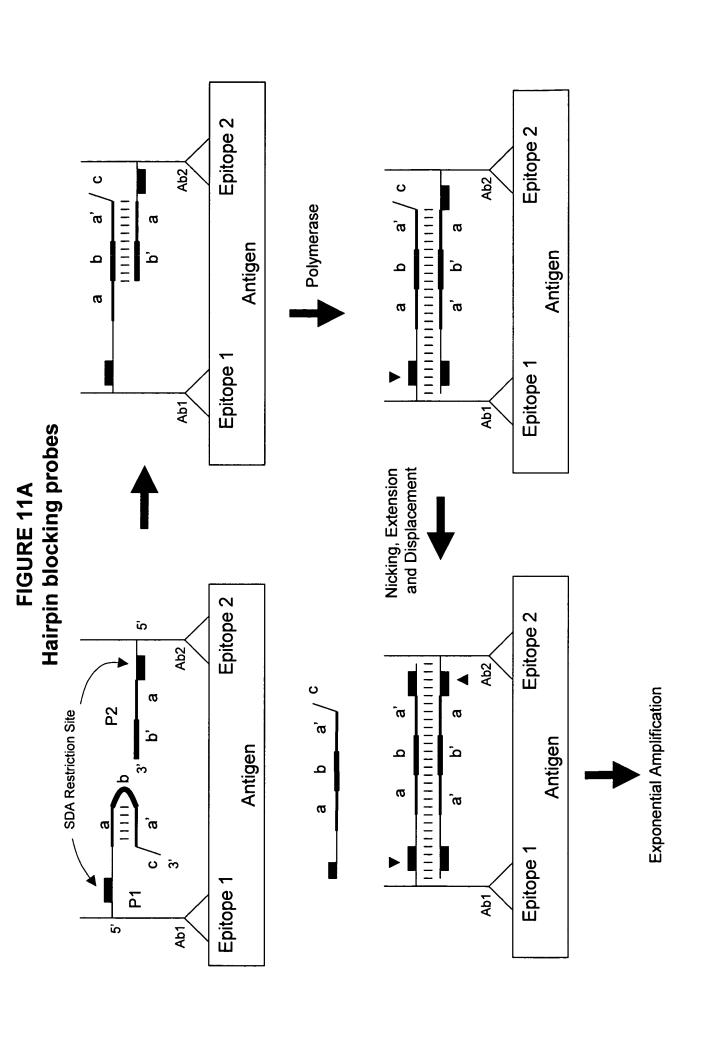
7 P2 7 FIGURE 8C: Heterogeneous immuno-amplification Ab1 Epitope 2 7 P2 Antigen 7 Epitope 1 Epitope 2 P2 Antigen 7 Capture and wash Epitope 1 P2 Epitope 2 7 Amplification P2 Antigen 7 Ab2 Epitope 1 **P**2

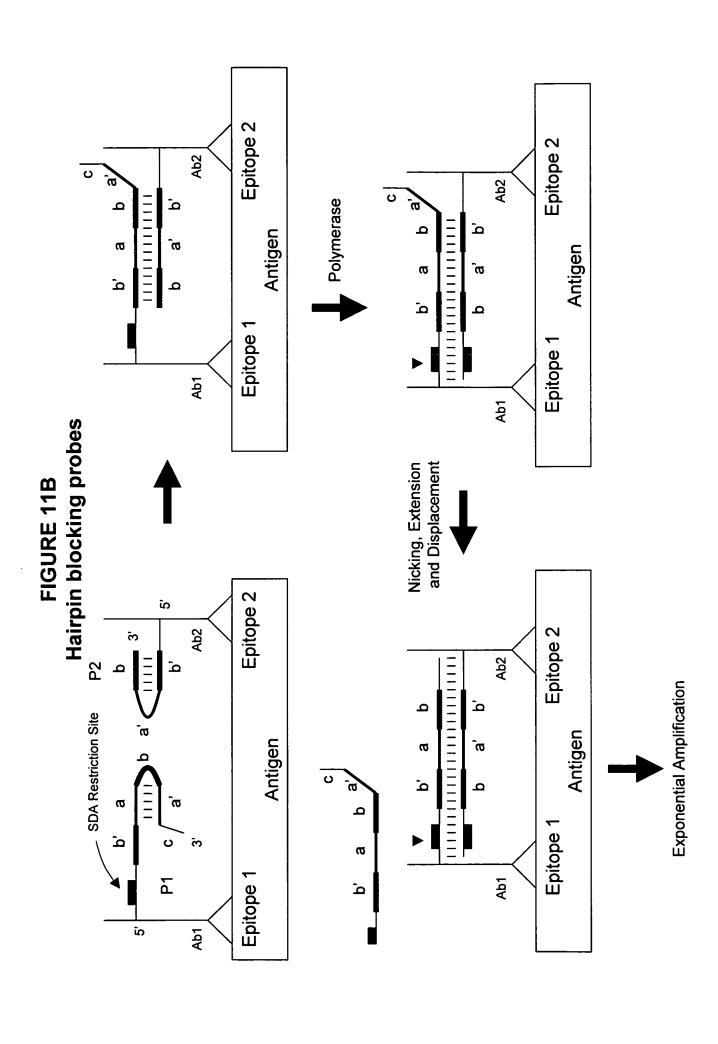
 ▼ Cleave Scissile
 ■ Cleave Scis Linkage Epitope 2 Epitope 2 Heterogeneous immuno-amplification with scissile linkage P2 Antigen Antigen 7 Epitope 1 Ab1 7 Epitope 1 Ab1 remove unbound Ab1 Wash to Transfer liquid phase **FIGURE 8D** 9 7 Epitope 2 Ab2 Amplification P2 Epitope 2 Antigen 7 P2 Epitope 1 Antigen Ab1 7 Epitope 1 Ab1

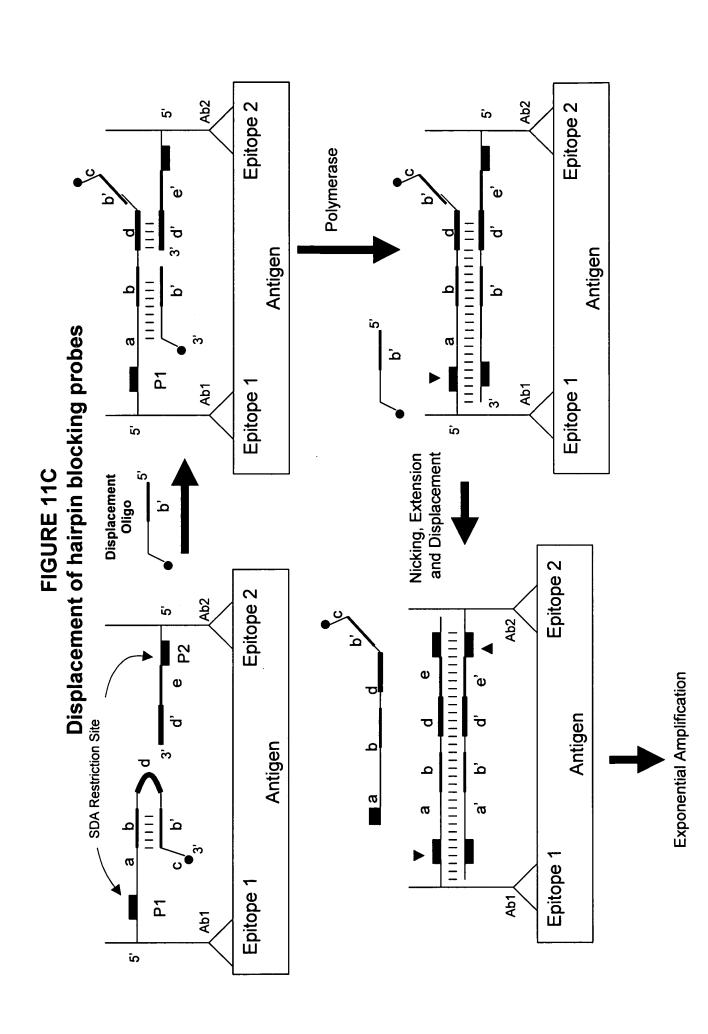
Heterogeneous immuno-amplification with linker oligonucleotides FIGURE 9

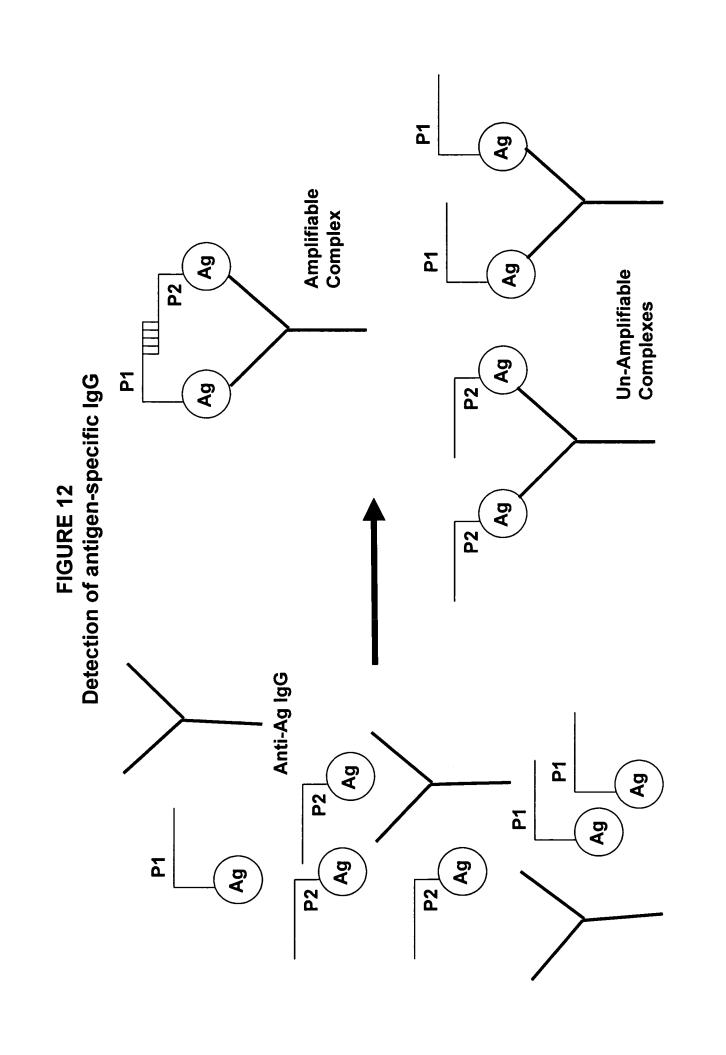


Sec 2 Polymerase Epitope 2 Epitope 2 P2 Antigen Antigen ιΞ' Universal immuno-amplification system Epitope 1 Epitope 1 7 Sec1 FIGURE 10 Add Universal Oligonucleotide-Iabeled anti-F_c Antibodies Amplification Epitope 2 Antigen Epitope 1

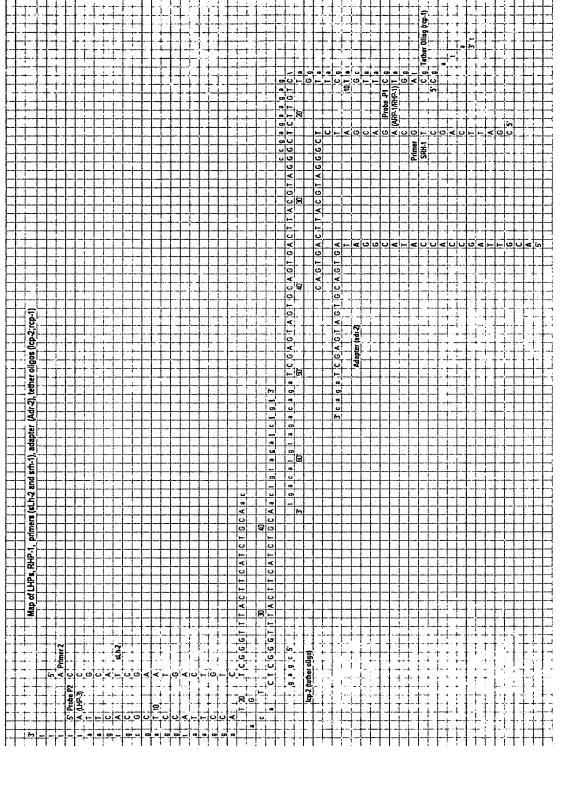




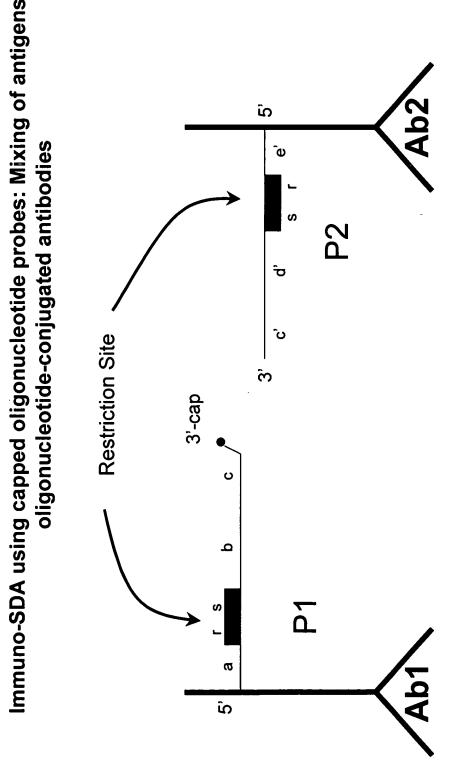




Map of probes, primers, tether oligos for binary immuno-SDA FIGURE 13



Immuno-SDA using capped oligonucleotide probes: Mixing of antigens and **FIGURE 14A**



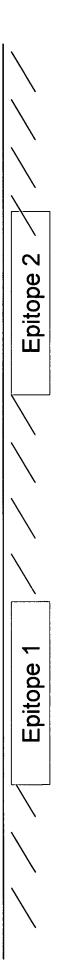
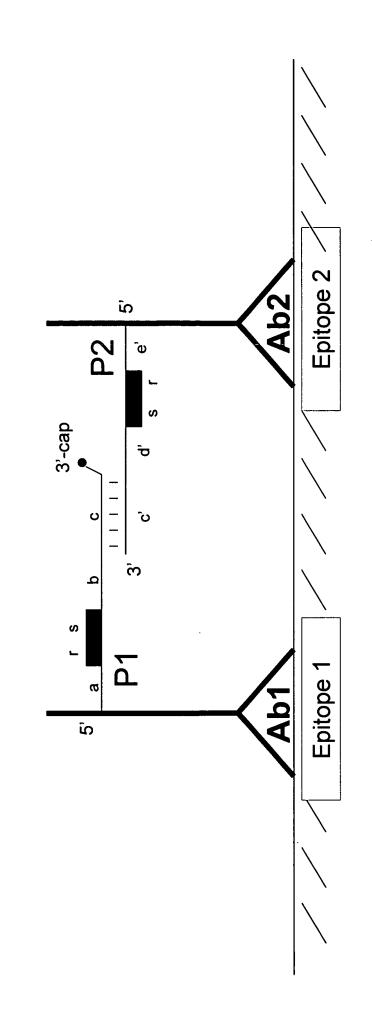
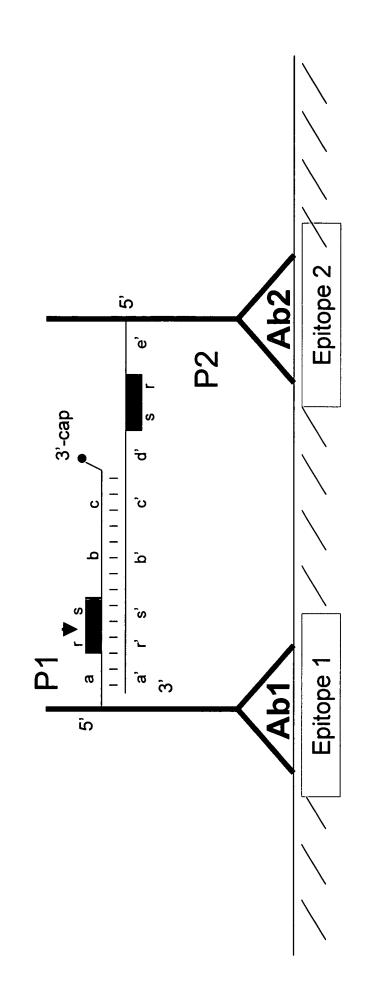


FIGURE 14B
Hybridization of adjacent probes



Polymerase extension and restriction enzyme nicking **FIGURE 14C**



Extension from nick and displacement of 3'-capped fragment **FIGURE 14D**

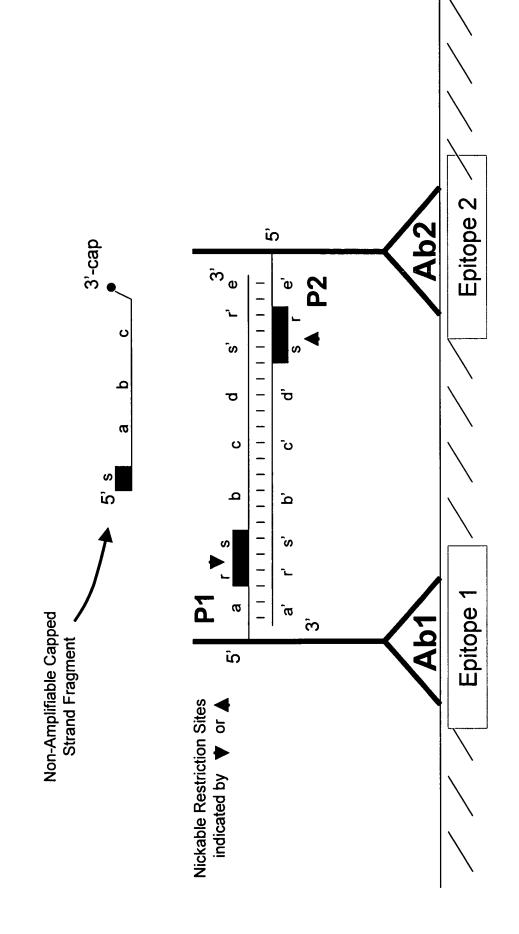
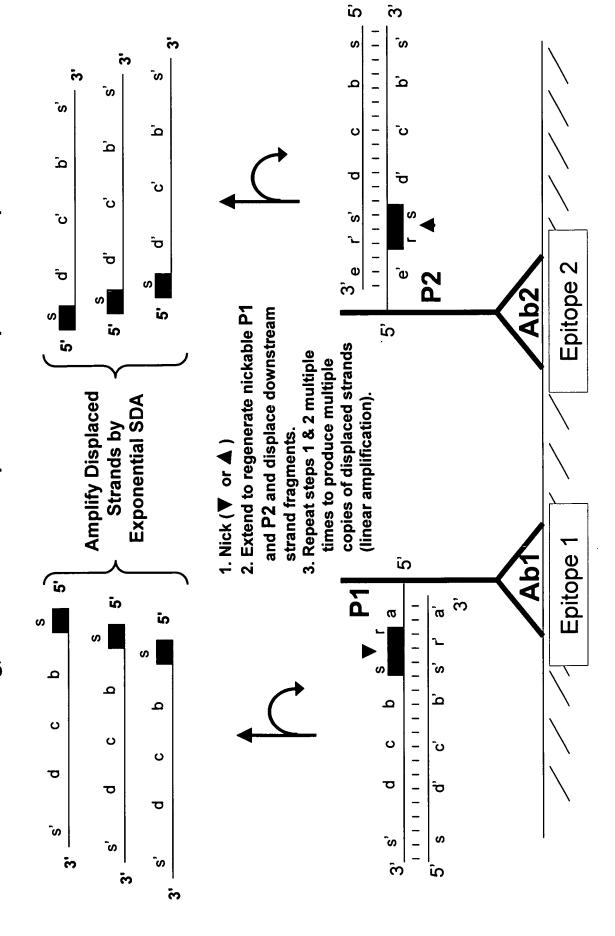


FIGURE 14E: Nicking, extension and displacement to produce amplifiable strands



Two-color, real-time fluorescence profile for immuno-SDA detection of IL-8 **FIGURE 15A**

